



CEDAR 038135

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In Re the application of:

RAMEZ E. SHEHADA, ET AL.

Serial No.: 08/889,017

Filed: July 7, 1997

For: METHOD AND DEVICES FOR LASER
INDUCED FLUORESCENCE
ATTENUATION SPECTROSCOPY

) Examiner: A. Israel

) Group Art Unit: 2878

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DECLARATION OF RAMEZ E. SHEHADA, PH.D.

Assistant Commissioner of Patents

Washington, D.C. 20231

I, RAMEZ E. SHEHADA, declare as follows:

1. I am a named inventor in the above-identified application.
2. I have a doctorate degree in Biomedical Engineering and have been actively conducting research in the fields of Biomedical Optics and Lasers for the past 9 years.
3. I have followed the prosecution of the above-identified application and have examined the Office Action of August 16, 1999 and the references cited in this application, including U.S. Patent Nos. 5,495,850 (Zuckerman) and 5,635,402 (Alfano et al.) and International Patent Application WO 97/08538 (Sevick-Muraca et al.).
4. In view of my study of Zuckerman, Alfano et al. and Sevick-Muraca et al., I set forth my observations in support of the Amendment submitted in response to the Office Action of August 16, 1999, to overcome the rejections under Sections 102 and 103.

[5] "The vector components $I_{||}$ and I_{\perp} are respectively and simultaneously detected by two optical detectors 88A and 88B, which can be photodiodes or photomultiplier tubes." See [column 10, lines 45-48]

Hence, the detectors 88A and 88B do not detect the fluorescence of the irradiated sample from a first and second distances from the sample, however, they detect the linearly polarized vector components $I_{||}$ (parallel 86A) and I_{\perp} (perpendicular 86B) of the fluorescence returned from a single location, which is the probe's tip 68.

[6] "Wavelengths >400 nm pass through the dichroic mirror 62 and wavelengths from 400-420 nm pass through an emission filter 82 to a Wollaston prism polarizer 84, which resolves the emitted fluorescence into its linearly polarized components parallel 86A and perpendicular 86B to the plane of excitation polarization." See [column 10, lines 40-45]

Upon reaching the apparatus, the returned fluorescence is resolved into its linearly polarized components parallel 86A and perpendicular 86B by the Wollaston prism polarizer 84.

[7] "Since optics are reversible, the linearly polarized fluorescence emission from the probe tip 68 returns along the single mode polarization-preserving glass fiber and is collected by the objective lens 64." See [column 10, lines 37-40].

Hence, the emitted fluorescence is collected from the probe tip 68 by a single optical fiber 66. Therefore, the fluorescence is not collected at first and second distances from the sample.

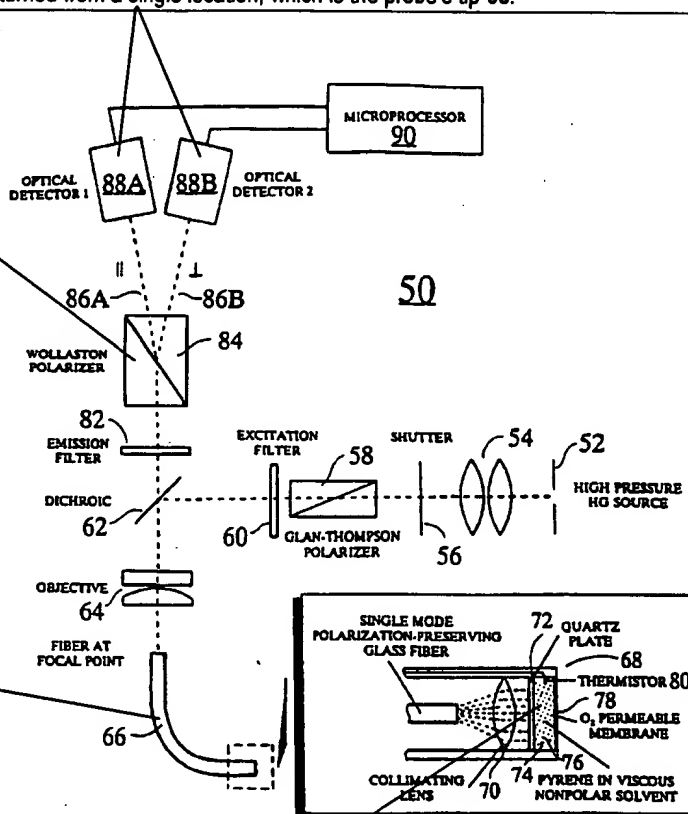


Figure 3

[8] "In the case of a narrow submillimeter catheter, the linearly polarized light passes directly to the O_2 -sensitive tip 68, whereas for larger diameter catheters the glass fiber 66 is mounted at the focal point of a collimating lens 70 which is used to provide uniform illumination of the catheter tip 68." See [column 10, lines 16-21].

"The catheter tip 68 contains a nonpolar viscous medium 74 such as paraffin or mineral oils in which pyrenebutyric acid or pyrene 76 is dissolved. Pyrene has a fluorescence lifetime approximately ten times that of pyrenebutyric acid or its salt form, thereby increasing the precision of the measurement, with an accuracy of PO_2 measurement of <1 mm Hg possible. The O_2 -quenchable probe substance dissolved in a nonpolar viscous solvent is sealed within the catheter by an O_2 permeable membrane 78 such as polyethylene." See [column 10, lines 24-34].

Hence, the polarized excitation radiation does not excite the sample itself, however, it excites a volume of an O_2 -quenchable fluorescent probe substance 76 (i.e. pyrenebutyric acid or pyrene) encapsulated in the probe's tip and separated from the sample by an O_2 permeable membrane 78. Therefore, the returned fluorescence is not measured from the sample at all, however, from single location within the tip 68 of the catheter.

Figure 3 of Patent number 5,494,850 by R. Zuckerman

[9] "The light passing through filters 55 and 57 is detected by photomultiplier tubes 59 and 61, respectively, and converted into electrical signals, which are transmitted to electronics lock-in and then processed by computer 52" See [column 5, lines 64-67].

This indicates that the photomultiplier tubes 59 and 61 detect the fluorescence intensity returned through bundle legs 51 and 53 passing through filters 55 and 57, respectively. Both fluorescence portions measured by the photomultipliers 59 and 61 were collected from a single spatial location facing the sample, which is which is the probe end 48 on the sample S.

[10] "The light emitted from slide S is collected by probe end 48 of bundle 47 and then transmitted to a second leg 51 and a third leg 53 of bundle 47. Second leg 51 and third leg 53 of bundle 47 are equipped with filters 55 and 57, respectively, which function in the same manner as filters 17-1 and 17-2, respectively, of system 11." See [column 5, lines 58-63].

Hence, the returned fluorescence is collected from a single spatial location facing the sample (i.e. the probe end 48) and then split into two portions for the purpose of filtration with two different filters. There is no mention whatsoever of a first and second waveguides disposed at a first and second distances distance from the sample.

[11] "X-Y movement of slide S to permit scanning of a larger area of the sample is effected by a motorized device 50, which is controlled by a computer 52." See [column 5, lines 54-57].

Each scan of a given location is processed separately and independently from preceding and subsequent scans performed on neighboring locations. The X-Y movement is performed only to scan a larger area of the sample because the system can inspect a given location (i.e. whatever is facing the "probe end" 48) at a given time.

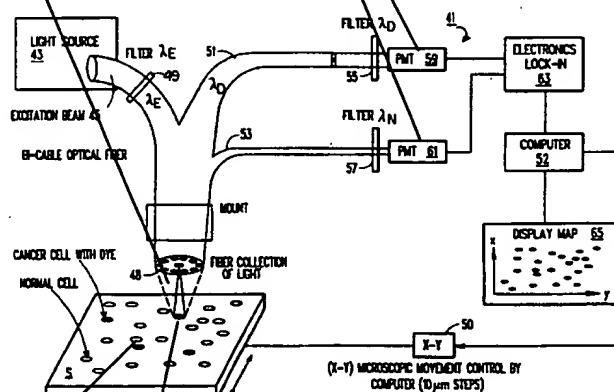


FIG. 2

[12] "The light transmitted from light source 43 is sent through a first leg 45 of a trifurcated fiber optic bundle 47. Disposed within first leg 45 is a filter 49, which is selective for light of a wavelength which will cause the dye in the sample to fluoresce, i.e. at a wavelength within the absorption curve for the dye." See [column 5, lines 39-44]. "The light emergent from the probe end 48 of bundle 47 illuminates a small portion of the slide S upon which the sample is smeared." See [column 5, lines 53-55].

Figure 2 of Patent number 5,635,402 by R. Alfano et al.

[13] "Fluorescent light emitted in response to the excitation light is detected with sensor (148). The AC intensity and phase of the excitation and detected fluorescent light is provided to a processor (160) operatively coupled to sensor (148). Processor (160) employs the measured re-emission kinetics of excitation and fluorescent light to "map" the spatial variation of one or more fluorescence characteristics of the tissue (100)." See Abstract [Lines 4-11]

[14] "System 110 also includes detection subsystem 140 which has optic fibers 143 to detect photons emitted from tissue 100 from a number of corresponding detection sites." See [page 7, lines 21-22].

"Sensors 128, 148 and source 120 are operatively coupled to heterodyne subsystem 130. Subsystem 130 is configured to obtain information about the phase, AC, and DC intensity of light detected with sensor 128 relative to light detected with the sensor 148 using conventional laser heterodyning techniques." See [page 8, lines 1-4].
 "In stage 216, the phase, θ_{obs} , and log of AC intensity, M_{obs} , of the emission at each detection site "i" relative to the excitation light from source 120 are determined at the heterodyne (or offset) frequency". See [page 9, lines 8-10].

Hence, the emission from each detection site is only processed relative to the source light detected by sensor 128, however, separately and independently from the emissions of other detection sites. The only purpose for Sevick-Muraca to use multiple detection sites is to determine the spatial variation (or image) of the (1) quantum yield and the (2) lifetime of the fluorescence throughout the sample.

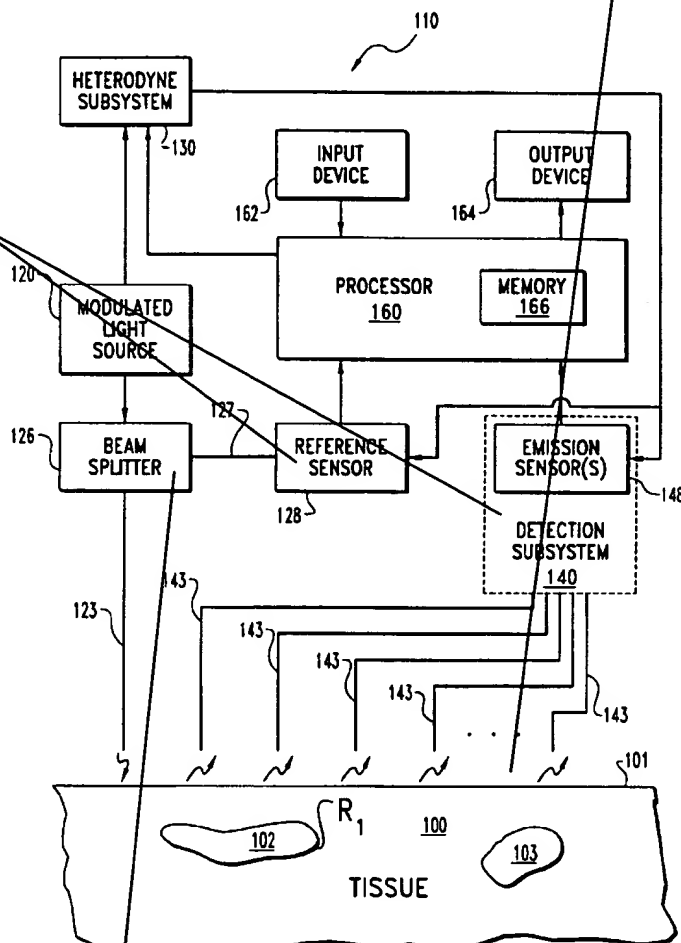


Fig. 1

[15] "Beam splitter 126 may be employed to direct a small portion of the excitation signal to reference sensor 128 for processing purposes." See [page 7, lines 19-20].

Hence, the excitation light is detected by sensor 128 to be used as reference by the heterodyne subsystem.

Figure 1 of Patent number WO 97/08538 by Sevick-Muraca

16. The instant claimed invention, in my opinion, is patentable over the cited references. None of the cited references discloses or even remotely suggests a method or an apparatus using modulated fluorescence of a sample measured at two different locations from the sample, for comparison to each other to determine a modulation characteristic of the sample.

The undersigned, being hereby warned that willful false statements and the like so made are punishable by fine or imprisonment or both, under 18 U.S.C. § 1001, and that such willful false statements may jeopardize the validity of the application or any resulting patent issuing thereon, declares that all statements made on information and belief are believed to be true.

2 / 16 / 2000
Dated

Ramez Shehada
Ramez Shehada, Ph.D.